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Influence of the Time of Acidification after Sample Collection on the Preservation of Drinking Water for Lead Determination

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16. ABSTRACT

It has been suggested that to obtain accurate data for trace metal analysis of drinking water, immediate acidification at the time of collection is necessary. However, because of problems encountered by EPA in field operations and epidemiology studies, further investigation into this requirement was pursued.

Drinking waters containing known concentrations of lead were acidified at different time intervals after collection. The water samples were then stored at room temperature for varying time intervals before analysis for lead. For each time period there were seven samples with three aliquots from each taken for analysis in duplicate by flameless Atomic Absrption Spectrophotometry (AAS).

All samples that were acidified gave a higher lead value than those samples containing lead but were not acidified. There were lead recovery variabilities among the time periods studies, however, it was evident that acidification of water samples after collection could be delayed up to 14 days without any adverse effect on lead concentration data.

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JOURNAL ARTICLE

Influence of the Time of Acidification after Sample Collection on the Preservation of Drinking Water for Lead Determination

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It has been suggested that to obtain accurate data for trace metal analysis of drinking water, immediate acidification at the time of collection is necessary. However, because of problems encountered by EPA in field operations and epidemiology studies, further investigation into this requirement was pursued. Drinking water samples containing known concentrations of lead were acidified at different time intervals after collection. The water samples were then stored at room temperature for varying time intervals before being analyzed for lead. For each time period there were seven samples with three aliquots from each taken for analysis in duplicate by flameless atomic absorption spectrophotometry (AAS). All acidified samples that were spiked with lead gave a higher lead value than the unacidified samples having comparable lead concentrations. Lead recovery varied among the time periods studied: however, the results indicated that acidification of water samples after collection could be delayed up to 14 days without any adverse effect on lead concentration data. This would allow acidification after samples are received in the laboratory and thus facilitate field studies.

Issaq and Zielinski (*1*), by use of flameless atomic absorption, reported on the need for immediate preservation of water samples after collection by comparing the loss of lead from aqueous solutions stored in glass or polyethylene containers. However, no data were reported on the lead concentration variability in those samples preserved immediately or at some other time interval after collection.

During some epidemiological studies investigating the health effects resulting from exposure to certain trace elements in drinking water, problems were encountered in complying with prescribed provisions for collection, preservation, and storage of drinking water samples (*2*). The recommeded U.S. Environmental Protection Agency procedure is that water samples collected for trace metal analysis should be acidified to pH <2 with HNO_3 at the time of collection or refrigerated for immediate shipment to the laboratory followed by acidification immediately upon arrival at the laboratory (*2*). Several factors have rendered these requirements of sample collection and preservation nearly unattainable in most population exposure studies which require a large number of samples; first, these studies require volunteer sample collection in homes by the general populace who are not familiar with the safety precautions necessary in handling the acid used in sample preservation; second, there are difficulties in meeting the U.S. Department of Transportation's regulation on the shipment of acid (corrosive material) because a vial of acid would have to be sent to each participant in the study; finally, it is impractical to arrange for the refrigeration of individual nonacidified samples before being sent to the laboratory for acidification and analyses.

Because of the acid handling problem associated with the sample collection, Guest and Blutstein (*3*) attempted to establish a different approach to adding acid preservative to water samples collected in the field. They inserted a sealed glass ampule containing acid into the sample collection bottle with the ampule being broken by shaking just prior to sample collection. This procedure did not take into consideration either the difficulty of transporting and shipping of the individual containers which are prone to breakage and/or contamination problems.

Sharrett et al. (*4*) reported on the methodology variations of lead analysis in drinking water but did not report on the effects of the sample and container interaction before acidification.

This research aims at improving the reliability of trace metals analysis in water samples which is needed to estimate human exposures to trace elements in toxicological and epidemiological studies and for regulatory purposes. Uncertainties of the time of acidification after collection of water samples have raised questions about the accuracy of their trace metal concentrations. Therefore, this study was designed to determine if preservation of water samples (by acid addition)

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immediately at the time of collection is necessary and to determine the time interval after sample collection that acidification may be delayed and still obtain valid analytical trace metal concentration data. Also, the question as to how long after a delayed acidification samples must be stored in order to attain their original trace metal concentration was explored.

Lead was chosen for this study due to the widespread health concerns regarding trace metals (i.e., lead) in drinking water and because of its believed instability associated with acidification time at collection and during storage.

EXPERIMENTAL SECTION

Experimental Design. A preliminary study using drinking water at two different lead concentrations was undertaken to determine the variability of data within and between sample containers and the variability of the analytical methodology. In the preliminary study, seven samples (1-L collapsible polyethylene sample container) were used for the blanks and seven samples for each of two lead concentrations, 26 and 105 μg/L. In the 0 and 26 μg/L groups of containers, six aliquots were analyzed in duplicate from each container while in the 105 μg/L group, 11 aliquots were analyzed in duplicate from each container. These numbers of replicates were based on single laboratory precision and accuracy data published by EPA (*2*) and chosen to produce reliable results at each concentration level. According to the statistical procedures of Cox (*5*), these data were used to estimate the standard error of differences between two sample means for each concentration. On the basis of precision data from the preliminary study and the limits as to the number of analyses that can be performed by one analyst within 1 day, it was determined that three aliquots analyzed in duplicate from each of seven samples at each sampling time would result in a statistically sound experiment (i.e., sufficient measurement of precision and statistical power to detect a true difference among acidification groups).

In the experiment, there were ten separate treatment groups of samples with seven samples in each group. These groups included acidified and nonacidified tap water used for background, nonacidified experimental water containing lead, and groups with experimental water containing lead which were acidified after collection at the time intervals of 0 min, 5 min, 6 h, 1 day, 3 days, 7 days, and 14 days. The time of analysis after acidification varied with each group but ranged from a few hours to several weeks.

The final experimental design included two levels of lead (20 μg/L and 70 μg/L). These concentrations of lead were used to represent water samples containing twice and half, respectively, of the EPA Maximum Contaminant Level for lead which is 50 μg/L.

The experimental water samples containing 70 and 20 μg/L lead from which the sample containers were filled were prepared in two 378-L stainless steel vats by circulating Cincinnati tap water via a pump through a plastic PVC pipe loop which contained a 35.6 cm section of 0.5 cm diameter lead pipe (*6*). The lead was leached from the lead pipe simulating lead dissolution in drinking water as it occurs in homes using plumbing products containing lead. The water was recirculated and routinely sampled until the desired level of lead was obtained at which time the lead pipe was removed but the water was continually recirculated. The vats were kept covered throughout the experiment with a floating lid which allowed no air space and thereby prohibited contamination or changes in pH which may influence lead solubility.

As the experimental vats were being filled, Cincinnati tap water was also being collected in a 37.8-L polyethylene container, acidified to pH <2 with HNO_3, and used as a diluent for the calibration curve. For comparison, an acidified tap water sample containing 33 μg/L lead was stored in a container made of Teflon and used as an undiluted standard to account for any day to day variability.

Analysis. Lead determinations were performed according to Perkin-Elmer Methods Manual (*7*, *8*) by flameless atomic absorption spectrophotometry with deuterium background correction. Instrumentation used was a Model 305A Perkin-Elmer atomic absorption spectrophotometer with a HGA 400 graphite furnace, an AS-1 autosampler, and a Model 56 recorder. Pyrolyzed graphite tubes coated with molybenum, as reported by Henn (*9*), were used to minimize carbide formation in the graphite tube. At the 20 μg/L level, a ramp time of 1 s was used during atomization; for the higher concentration of 70 μg/L, the ramp time was set at 0 s.

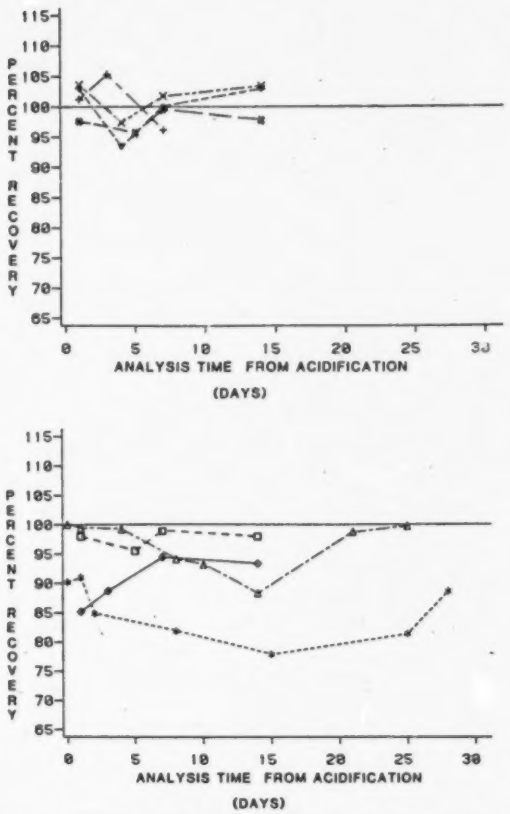

Figure 1. Stability of 70 μg/L Pb level vs. the time from sample acidification: (top) #, 5 min; +, 24 h; ×, 3 days; ▼, 7 days; (bottom) △, 0 min; □, 6 h; ◇, 14 days; •, no acidification.

In the preiminary study of the analytical methodology, calibration standards were prepared in the Cincinnati tap water control and compared with the calibration standards prepared in distilled/deionized water. A linear regression routine was performed on the resulting calibration curves. The control Cincinnati tap water yielded a slope of 0.5786 compared to a slope of 0.6752 in distilled/deionized water, thus indicating the presence of a negative matrix interference in the tap water. The experimental Cincinnati tap water was analyzed for the cations and anions known to interfere with lead analysis and the concentrations were found to be well below those at which there is a significant interference at these experimental lead levels. But to ensure experimental data reliability, the standard calibration curves for lead were prepared by matrix matching using the previously collected Cincinnati tap water control. This control water contained less than the detectable limit of 2 μg/L lead.

The pH values of the experimental waters ranged from 7.45 to 7.99 for the duration of the experiment.

RESULTS AND DISCUSSION

Nested analysis of variance procedures (*10*) were used to examine the data for each lead level for differences among the acidification groups and change in lead level over time of analysis. The factors included in the analysis were acidification group, sample container, analysis time, and aliquot. All sample groups of the 70 μg/L and 20 μg/L lead levels were related to their respective initial control value (Figure 1 and Figure 2). An examination of the data revealed only one consistent pattern in lead recovery over time; the nonacidified groups consistently attained less of their original values than

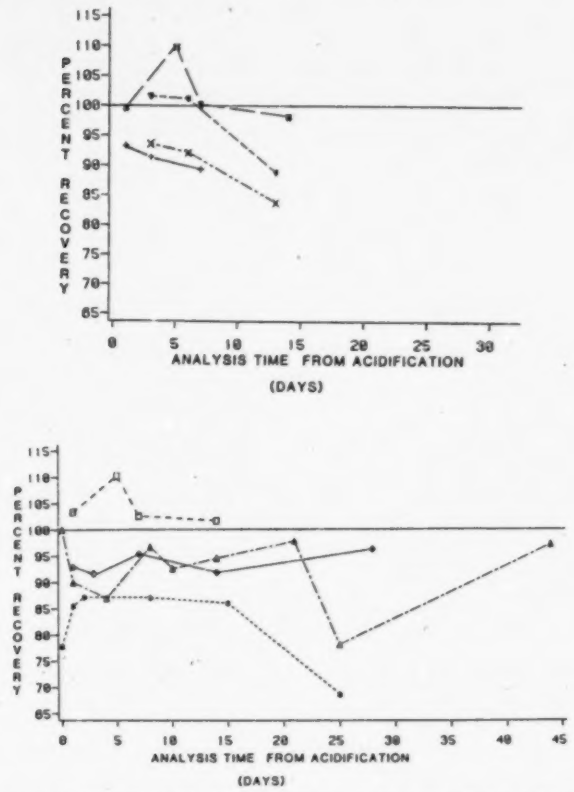

Figure 2. Stability of 20 μg/L Pb when comparing recoveries with time of acidification: (top) #, 5 min; +, 24 h; ×, 3 days; ▼, 7 days; (bottom) △, 0 min; □, 6 h; ◇, 14 days; *, no acidification.

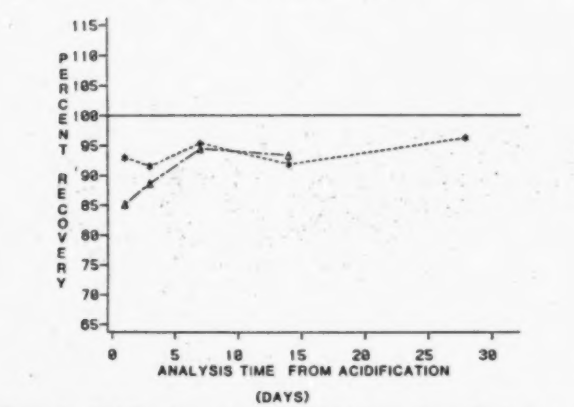

Figure 3. Stability of samples acidified 14 days after collection: △, 70 μg/L Pb; *, 20 μg/L Pb.

any of the other groups. The differences in the stability of the 3 day, 7 day, and 24 h groups is due to the day to day variability within the measurement system since all samples could not be analyzed on the same day.

For the two groups that were acidified 14 days after collection (Figure 3), the initial low recovery values obtained on the third day after the samples were acidified increased to nearly 95% of their expected values by the seventh day. There also was evidence that those groups acidified at 5 min and 6 h after collection and analyzed on the same days throughout the study gave similar lead concentration recovery data. (Figure 1 and Figure 2).

The results of the statistical analysis indicated highly significant differences ($p < 0.001$) among all factors except the sampe containers. Table I reports the overall group mean

Table I. Mean Percent Recovery with Respect to Control Value

acidification	mean % recovery[a] 70 μg/L	20 μg/L
no acidification	85	82
0 min	97	93
5 min[b]	98	102
6 h[b]	98	104
24 h	101	91
3 days	102	90
7 days	100	97
14 days	90	94

[a] The number of analyses per group varied (n ranged from 126 to 336). [b] Had same initial control value and always analyzed on same day.

Table II. Precision and Accuracy Estimates[a]

samples	overall mean	av std dev duplicates	aliquots
20 μg/L	21.13 (378)	0.37 (188)	0.76 (63)
control (33 μg/L)	32.97 (126)	0.37 (63)	0.90 (21)
70 μg/L	67.7 (376)	0.54 (168)	1.49 (56)

[a] Parentheses: number of samples (n).

percent recovery. No logical order is evident in the average readings at either lead level. In fact the order is not the same at the two lead levels. It should be noted that due to the logistics of conducting the study both sets of means are confounded with the date of analysis and time from acidification. Because of these problems and the interaction seen in the data, separate analyses were performed for each of the analysis time that had data from more than one acidification group. Again, highly significant differences ($p < 0.001$) among groups and among aliquots were found and no consistent pattern of differences among acidification groups could be ascertained.

The precision and accuracy of the analytical methodology were estimated using the control data (samples acidified immediately) from the 70 μg/L and 20 μg/L samples and a spiked control sample.

Because of the day to day variability of the overall method, on each day that the experimental samples were analyzed, three aliquots of the undiluted standard (33 μg/L) were analyzed in duplicate. Table II shows the overall mean and average standard deviations in micrograns per liter for all analyses of the control samples.

The percentage difference between a daily mean value for the spiked sample and the overall mean value for the spiked sample was used to calculate a daily correction factor. The correction factor, when applied to the appropriate daily lead concentration means of the 20 μg/L and 70 μg/L groups gave a better lead stability recovery data as shown for 0 min acidification vs. nonacidified samples at the 20 μg/L level (Figure 4). The comparison by ratio of the lead concentration values of the samples containing 70 μg/L lead and acidified immediately after collection to the corresponding group that was nonacidified but analyzed on the same days (Figure 5) gives a clear indication of the decrease in recovery of lead from nonacidified samples.

The data were reanalyzed using the calculated correction factor to adjust all daily readings. Although the overall mean values changed slightly and the amount of variability in the data decreased, there were no major differences in the results of the reanalysis. On the basis of these results and the variability of lead recovery in the control sample (acidified immediately), it was judged that the differences found among the acidified samples were indicative of the inherent variability

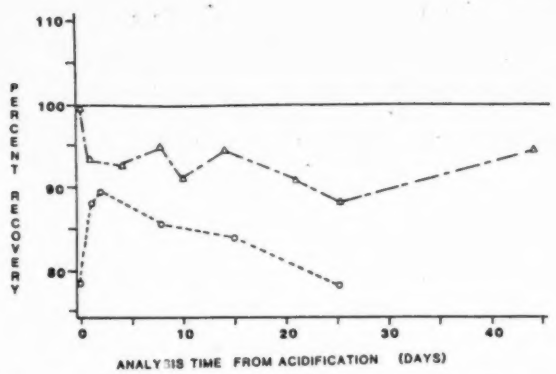

Figure 4. Percent recovery of immediately acidified group vs. nonacidified group after correction for daily method variability (20 μg/L level): O, not acidified; Δ, acidified immediately.

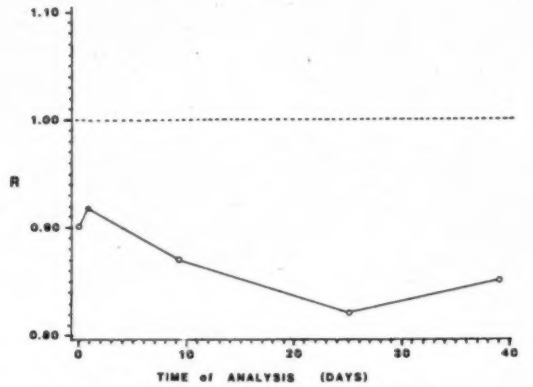

Figure 5. Concentration ratio of control group, acidified immediately, to corresponding nonacidified group (70 μg/L level). R = [nonacidified]/[control].

within the system and not of any true effect of acidification time.

CONCLUSIONS

Due to the inherent variabilities within the analytical system it is not possible to make a clear distinction between those groups acidified immediately and those acidified at 5 min, 6 h, 3 days, 7 days, and 14 days after sample collection. In general, all acidified samples had average lead recoveries within 10% of their initial control values throughout the study. Therefore, this study indicates that samples collected for lead analysis need not be acidified at the site of collection but must be acidified within 14 days after collection so as not to appreciably affect the experimental results. The data indicate that the analysis can be performed within a day after acidification provided acidification occurs within 14 days of collection.

Also, due to some unknown interferences in water analysis, this study shows the need for either matrix matching or methods of standard addition when analyzing unknown water for lead.

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